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13. ABSTRACT *(Maximum 200 Words)*
Segregation analyses of familial prostate cancer have provided evidence for the existence of dominantly-acting prostate cancer susceptibility alleles, with such genes being estimated to be responsible for about nine percent of all cases of prostate cancer in the U.S. These findings provided the basis for our genome wide scan for linkage in hereditary prostate cancer (HPC) families, leading to the identification of the *HPC1* locus at 1q24-25 as the first reported linkage in prostate cancer (Smith et al., *Science* 274:1371, 1996). Since this finding multiple other HPC loci have been identified, including our finding of the *HPCX* locus at Xq27-28 (Xu et al. *Nat. Gen.* 20:175, 1998). These results emphasize the genetic heterogeneity that characterizes HPC. To increase the power of our family collection in an effort to deal with this heterogeneity, we propose to collect an additional 57 HPC families, each having over 4 individuals affected with prostate cancer. To date we have collected 53 of these families and we have carried out genotypic analysis of these and our existing families at a series of putative HPC loci, including loci implicated by other research groups on chromosomes 1 and 8. Novel loci have been implicated on both of these chromosomes. By accumulating linkage data on our complete set of over 170 HPC families, we are able to begin to understand and evaluate genetic heterogeneity of HPC, as well as to provide critical positional information for gene mapping and identification studies. Such studies are prerequisite to the development of genetic tests for determination of prostate cancer susceptibility.

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Introduction

In spite of the magnitude of the problem which prostate cancer presents, our understanding of the molecular mechanisms underlying prostatic carcinogenesis remains elusive. It is clear from the recent progress made in colorectal, renal and breast cancer that analysis of familial forms of common human neoplasms can yield tremendous insight into the specific genetic mechanisms in both hereditary and sporadic forms of such cancers. Hereditary factors are estimated to be responsible for about nine percent of all cases of prostate cancer in the U.S. Segregation analysis of familial prostate cancer has supported an autosomal dominant mode of inheritance of prostate cancer susceptibility alleles with some evidence for heterogeneity. These findings provided the basis for a genome wide scan for linkage in multiplex prostate cancer families. This analysis implicated 1q24-25 as being the most likely region of the genome to contain a major prostate cancer susceptibility gene (HPC1). Interestingly, this evidence for linkage was provided almost exclusively by large families (5 or more first degree relatives affected/family) with an early average age of diagnosis (<65 years). However, there was significant evidence for locus heterogeneity and a series of other loci also showed evidence of linkage, albeit to a lesser extent than HPC1. *It is the goal of the research proposed herein to further analyze these other regions for evidence of linkage to prostate cancer susceptibility.* To detect these potential linkages, 57 additional families, each containing at least five affected members and over half having an average age of diagnosis under 65, will be collected for these studies, as deemed necessary from simulation analyses. Genotypic data for these families in the regions of interest will be analyzed using both parametric and non-parametric methods, including conditional analyses and two locus models to test for gene-gene interactions. These studies will provide the basis for positional cloning efforts to identify and characterize prostate cancer susceptibility genes.

Body

Progress report for Activities During Months 1-24

Listed below is a summary of the research objectives as described in the approved Statement of Work as it applies to the first 24 months of the funding period, along with the accomplishments pertaining to these objectives.

Task 1) ***Ascertain 57 additional families with at least 5 members with prostate cancer (months 1-30).***

Accomplishments related to Task 1: Within the first 24 months of Phase I, we ascertained 53 of the 57 families proposed in our specific aims. We contacted each living family member to obtain informed consent and blood DNA. Blood or tissue samples have been obtained from 248 individuals in these new families. The remaining 4 families will be collected before the end of Phase I. Tables I summarize the characteristics of our complete family collection of 171 HPC families, each having at least 3 first degree relatives with prostate cancer. Fifty-three % of these families have 5 or more first degree relatives affected.

Table I HPC Family Collection

	All Families (n=171)	Families with 5 or more Affected Men (n=94)
# Affected	839	502
Average # Affected per family	4.9	5.3
# Blood DNAs	1008	645
Average Age of Diagnosis per family	64.2	63.8
# Unaffected Males	271	177
# Females	379	273
# Affected with Age of Dx < 65	359	232

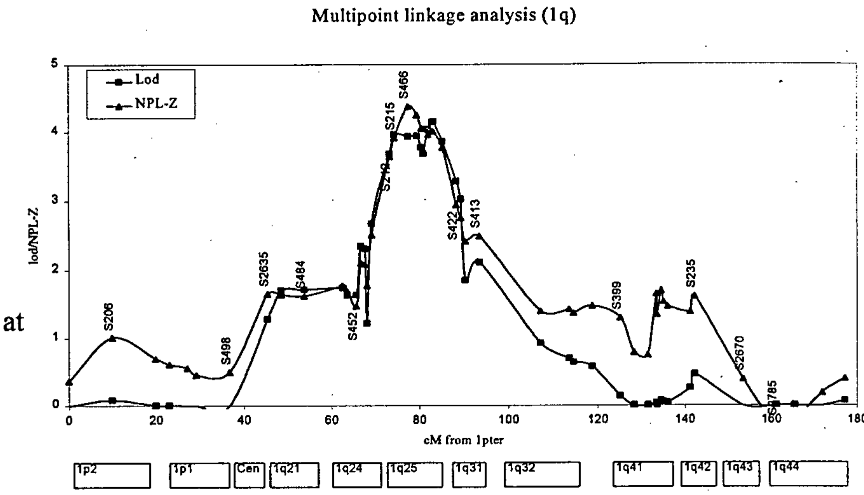
Task 2) ***Genotype the new and current sets of families for highly polymorphic markers in the chromosomal regions for which we have preliminary evidence of linkage (months 1-30).***

Accomplishments related to Task 2: Genotypes have been generated for over 1000 individuals in the existing 171 families for the following sets of markers: Xq27-28, 40 markers; 8p, 20 markers; 13q, 25 markers; 1q42-43, 6 markers; 1p36, 6 markers; 16 markers located in between 1p36, 1q24-25 and 1q42-43; 17p13, 6 markers, for a total of 119 loci. DNA has been prepared from 230 individuals in the 53 new families and a majority of these markers have been analyzed in this dataset.

Accomplishments related to Task 3: Analysis of marker data for chromosomes 1, X, and a novel locus on chromosome 8 are summarized here.

Figure 1
Summary Results for Chromosomes X and 1

1. Genotyping of markers spanning chromosome 1, with emphasis on *CaPB* at *1p36*, *PCaP* at *1q42-43*, and *HPC1* at *1q24-25*, reveals significant evidence of linkage only at *HPC1* in this collection of 159 HPC families (fig.1).



2. Evidence of linkage is observed at *Xq27-28* in 139 HPC families collected at Johns Hopkins (JHU) (Table 2) (Xu et al. 1998). In addition, evidence of linkage to this region is also observed in 123 HPC families collected at the Mayo Clinic and 57 HPC families collected at Tampere University, Finland.

Table 2 • Two-point parametric lod scores

Marker	Heterozygosity	cM[b]	JHU (139)	Mayo (123)[c]	lod (θ)[a] Tampere (57)	Umeå (41)[d]	All (360)
DXS984	0.74	140.0	0.40 (0.36)	0.31 (0.34)	0.87 (0.22)	0.03 (0.44)	1.00 (0.34)
DXS1232	0.66	140.9	0.28 (0.36)	0.00 (0.50)	0.66 (0.22)		0.24 (0.40)
DXS1205	0.66	142.3	0.19 (0.38)	0.00 (0.50)	2.05 (0.14)		0.33 (0.36)
DXS6751	0.74	143.6	0.49 (0.36)	0.52 (0.32)	1.56 (0.18)		1.63 (0.32)
DXS6798	0.83	144.8	0.51 (0.36)		0.78 (0.22)		0.87 (0.32)
DXS8106	0.70	146.1	0.82 (0.34)	0.80 (0.30)	0.89 (0.16)		1.93 (0.30)
DXS6806	0.81	147.3	0.45 (0.36)	0.78 (0.30)	0.14 (0.28)	0.03 (0.44)	1.07 (0.34)
DXS8043	0.83	148.8	0.97 (0.32)	0.02 (0.40)	0.00 (0.50)	0.08 (0.38)	0.74 (0.36)
AFMA113zf5	0.68	149.3	0.11 (0.36)	1.24 (0.28)	1.22 (0.18)		2.01 (0.28)
DXS1200	0.60	150.4	1.98 (0.28)	0.86 (0.26)	0.17 (0.32)	0.00 (0.50)	2.80 (0.30)
DXS297	0.70	151.0	0.64 (0.34)	0.18 (0.36)	0.13 (0.00)		0.84 (0.34)
AFM136yb10	0.68	152.5	1.00 (0.30)	0.40 (0.30)	0.05 (0.38)		1.38 (0.32)
DXS8091	0.80	152.5	1.52 (0.30)	0.28 (0.34)	0.00 (0.50)		1.43 (0.32)
DXS1113	0.80	153.0	1.73 (0.28)	1.89 (0.26)	0.49 (0.22)	0.60 (0.26)	4.60 (0.26)
DXS1193	0.78	153.3	0.96 (0.32)		0.58 (0.26)	0.34 (0.32)	1.80 (0.30)
DXS8069	0.67	154.5	0.44 (0.36)	0.84 (0.30)	0.01 (0.40)	0.12 (0.38)	1.20 (0.34)
DXS8011	0.87	154.6	0.32 (0.36)		0.58 (0.26)		0.72 (0.34)
DXS8103	0.77	155.2	0.10 (0.42)	0.38 (0.34)	0.92 (0.24)	0.29 (0.32)	1.10 (0.36)
AFMA225xh9	0.74	156.3	0.31 (0.36)	0.98 (0.30)	0.00 (0.50)		0.68 (0.36)
AFMA08xa5	0.51	157.1	0.02 (0.44)	0.02 (0.40)	0.09 (0.00)		0.03 (0.42)
DXS1108	0.70	158.8	0.12 (0.42)	0.57 (0.32)	0.00 (0.50)		0.42 (0.38)

[a]Maximum lod score under homogeneity with the maximum likelihood estimate of the recombination fraction (θ), calculated using FASTLINK. [b]Distance in cM from Xpter. [c]Three markers were not genotyped in this group. [d]Thirteen markers were not genotyped in this group.

Summary Results for Chromosome 8

Background for studying 8p

1. An elevated lod score was observed in our initial analysis of 66 HPC families. The lod scores was 1.24 at D8S550.
2. Many LOH have demonstrated frequent inactivation of one of more prostate tumor suppressor genes on 8p, but these genes remain unidentified.
3. A French biotech company (GENSET), led by Daniel Cohen, identified a putative prostate cancer susceptibility gene (PG1) at 8p23, described in US Patent #5,945,522 (8/31/99).

Purpose

a. To investigate the linkage between prostate cancer susceptibility gene and 8p markers in 159 HPC families
b. To examine linkage to PG1 using both family-based association method in 159 HPC families and population-based association method in 159 HPC cases, 47 sporadic cases and 91 controls. Since parental genotype data is usually unavailable in prostate cancer sample, we used the Reconstruction-combined Transmission Disequilibrium Test (RC-TDT) method for the family-based association test. The RC-TDT systematically reconstructs parental genotype based on offspring data and combined with sib-pair TDT. This approach has better power to detect association and linkage.

Results

1. Evidence for linkage was observed in the 159 HPC families. The peak lod score assuming heterogeneity was 1.91 at marker 17 (Table 3). The peak NPL score was 2.68 (p=0.004) at the same marker. These values meet the Lander-Kruglyak criteria for suggestive linkage (Nature Genetics 11:241-247, 1995).
2. The evidence for linkage in 11 Ashkenazi families was stronger. The proportion of families linked to this region ranged from 60% to 96% (Table 4).
3. There was no evidence for association between PG1 and prostate cancer using RC-TDT method in 159 HPC families. However, there were several markers at the peak linkage region with marginally significant p-values (Table 5)
4. There was no evidence for association between PG1 and prostate cancer using case-control analysis. There was no statistical difference in the SNPs allele frequency between 159 HPC cases, 47 sporadic cases, and 91 unaffected controls (Table 6). There was also no statistical difference in the haplotype frequency between cases and controls (Table 7).

Table 3. Multipoint Linkage Results at 8p Region in 159 HPC Families

	Multipoint		NPL	
	hlod	alpha	Z-score	P-value
Marker1	0.24	0.06	0.41	.33
Marker2	0.51	0.08	1.12	.13
Marker3	0.59	0.08	1.36	.08
Marker4	0.25	0.05	1.15	.12
Marker5	0.55	0.08	1.49	.07
PG1-a	0.60	0.08	1.51	.07
PG1-b	0.60	0.08	1.51	.07
PG1-c	0.60	0.08	1.54	.06
PG1-d	0.60	0.08	1.53	.06
PG1-e	0.61	0.08	1.55	.06
Marker11	0.68	0.08	1.88	.03
Marker12	1.19	0.12	2.56	.006
Marker13	1.19	0.12	2.50	.007
Marker14	1.14	0.12	2.28	.01
Marker15	1.26	0.12	2.33	.01
Marker16	1.25	0.12	2.39	.009
Marker17	1.91	0.15	2.68	.004
Marker18	1.10	0.12	2.48	.008
Marker19	1.23	0.12	2.68	.004
Marker20	0.47	0.08	1.30	.09

Table 4. Multipoint Linkage Results at 8p Region in Three Race/Ethnic Groups

marker	African American		Ashkanazi		Caucasian	
	alpha	HLOD	alpha	HLOD	alpha	HLOD
Marker1	0.00	0.00	0.45	0.44	0.07	0.23
Marker2	0.00	0.00	0.45	0.49	0.10	0.64
Marker3	0.00	0.00	0.48	0.59	0.10	0.71
Marker4	0.00	0.00	0.46	0.59	0.06	0.30
Marker5	0.00	0.00	0.44	0.56	0.09	0.66
PG1-a	0.00	0.00	0.46	0.54	0.09	0.64
PG1-b	0.00	0.00	0.47	0.55	0.09	0.64
PG1-c	0.00	0.00	0.47	0.56	0.09	0.64
PG1-d	0.00	0.00	0.48	0.57	0.09	0.64
PG1-e	0.00	0.00	0.49	0.58	0.09	0.64
Marker11	0.00	0.00	0.59	0.91	0.08	0.63
Marker12	0.00	0.00	0.60	1.00	0.12	1.12
Marker13	0.00	0.00	0.60	1.01	0.12	1.12
Marker14	0.00	0.00	0.60	1.03	0.12	1.06
Marker15	0.00	0.00	0.61	1.04	0.13	1.17
Marker16	0.00	0.00	0.61	1.05	0.13	1.16
Marker17	0.00	0.00	0.62	1.06	0.15	1.82
Marker18	0.00	0.00	0.66	1.10	0.12	0.97
Marker19	0.20	0.49	0.72	1.14	0.09.	0.48
Marker20	0.27	0.64	0.96	1.24	0.02	0.01

5. These results indicate the presence of an HPC gene on chromosome 8, but effectively exclude PG1 as being this gene.

Table 5. Reconstructed-combined TDT at 8p Region

	Allele	P_exact	P_Z
Marker1	9	0.008	0.006
Marker2	6	0.05	0.04
Marker3	Any	n.s.	n.s.
Marker4	Any	n.s.	n.s.
Marker5	Any	n.s.	n.s.
PG1-a	Any	n.s.	n.s.
PG1-b	G	n.s.	n.s.
PG1-c	C	n.s.	n.s.
PG1-d	Any	n.s.	n.s.
PG1-e	T	n.s.	n.s.
Marker11	5	0.03	0.02
Marker12	Any	n.s.	n.s.
Marker13	Any	n.s.	n.s.
Marker14	4	0.04	0.03
Marker15	8	0.04	0.02
Marker16	11	0.06	0.04
Marker17	Any	n.s.	n.s.
Marker18	7	0.03	0.03
Marker19	14	0.03	0.03
Marker20	Any	n.s.	n.s.

Table 6. Allele frequencies of SNPs in PG1

	# of sample	PG1-b allele 'G'	PG1-e allele 'T'
Controls	91	0.25	0.21
All cases	218	0.31	0.24
Sporadic cases	47	0.34	0.27
HPC cases	171	0.28	0.23
HPC cases, lod >0	45	0.3	0.24

The difference in the allele frequencies is not statistically significant

Table 7. Haplotype frequencies of SNPs in PG1

	# of sample	PG1-b and PG1-e ('T-G')
Controls	91	0.185
All cases	218	0.208
Sporadic cases	47	0.207
HPC cases	171	0.209
HPC cases, lod >0	45	0.22

The difference in the haplotype frequencies is not statistically significa

Key Research Accomplishments

- ascertainment of 53 new HPC families, with an average of 4.8 prostate cases per family
- collection of blood samples form 248 individuals in these families, and the preparation of DNA and lymphoblastoid cell lines from these individuals
- genotyping of 119 marker loci on our existing family collection and a subset of the newly ascertained families
- two-point and multipoint linkage analyses of these data are largely complete
- novel loci have been implicated on 8p, and 1p

Reportable Outcomes

- manuscripts
    - Xu et al. *Nat. Gen.* 20:175, 1998 Evidence for a Prostate Cancer Susceptibility Locus on the X Chromosome.
    - Xu et al. *Am J Hum Genet* Mar;66 (3):945-57, 2000. Combined Analysis of Hereditary Prostate Cancer Linkage to 1q24-25: Results from 772 Hereditary Prostate Cancer Families from the International Consortium for Prostate Cancer Genetics.
    - Jianfeng Xu, Siqun L. Zheng, Bao-li Chang, Jeffrey R. Smith, John D. Carpten, O. Colin Stine, Sarah D. Isaacs, Kathy Wiley, Lauren Henning, Charles Ewing, Piroska Bujnovszky, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Linkage of prostate cancer susceptibility loci to chromosome 1. Submitted.
    - Jianfeng Xu, Siqun L. Zheng, John D. Carpten, Nina N. Nupponen, Christiane Robbins, Juanita Mestre, Tracy Moses, Dennis Faith, Brian Kelly, Sarah D. Isaacs, Kathy Wiley, Bao-li Chang, Joan Bailey-Wilson, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Evaluation of linkage and association of HPC2/ELAC2 in familial and unrelated prostate cancer patients. Submitted.
    - Jianfeng Xu, Siqun L. Zheng, Bao-li Chang, Jeffrey R. Smith, John D. Carpten, O. Colin Stine, Sarah D. Isaacs, Kathy Wiley, Lauren Henning, Charles Ewing, Piroska Bujnovszky, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Identification of a novel HPC locus on chromosome 8. In preparation.

Conclusions

A cohort of 53 new hereditary prostate cancer families containing 253 affected men has been ascertained, and blood samples collected from 248 family members. These families combined with our previous collection provide a unique resource of 171 HPC families, highly informative for linkage analysis. Genotyping has been carried out on these families, and linkage analysis of these data performed. These analyses have led to the demonstration that, of all putative HPC loci identified to date, the three most important in our family collection are *HPC1*, *HPCX* and a novel locus on chromosome 8 (which is not PG1). These findings provide the basis for extended efforts to identify prostate cancer susceptibility genes and will greatly increase our ability to understand and characterize the genetic heterogeneity of hereditary prostate cancer. It is critical to understand this aspect of HPC if we are to develop meaningful genetic tests to identify individuals at high risk of developing this disease.

References

Smith et al., *Science* 274:1371, 1996
Xu et al. *Nat. Gen.* 20:175, 1998